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(54) Title: INSULIN PREPARATIONS CONTAINING CARBOHYDRATES

(57) Abstract

Insulin preparations of superior physical stability, comprising dissolved and/or precipitated human insulin or an analogue or derivative thereof, and a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof are disclosed.

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INSULIN PREPARATIONS CONTAINING CARBOHYDRATES

Introduction

The present invention relates to aqueous insulin preparations comprising human insulin or an analogue or derivative thereof, which preparations have superior physical stability. The invention furthermore relates to parenteral formulations comprising such insulin preparations and to a method for improving the physical stability of insulin preparations.

Background of the invention

Field of the invention

Diabetes is a general term for disorders in man having excessive urine excretion as in diabetes mellitus and diabetes insipidus. Diabetes mellitus is a metabolic disorder in which the ability to utilize glucose is more or less completely lost. About 2% of all people suffer from diabetes.

Since the introduction of insulin in the 1920's, continuos strides have been made to improve the treatment of diabetes mellitus. To help avoid extreme glycemia levels, diabetic patients often practice multiple injection therapy, whereby insulin is administered with each meal.

In the treatment of diabetes mellitus, many varieties of insulin preparations have been suggested and used, such as regular insulin, Semilente® insulin, isophane insulin, insulin zinc suspensions, protamine zinc insulin, and Ultralente® insulin. As diabetic patients are treated with insulin for several decades, there is a major need for safe and life quality improving insulin preparations. Some of the commercial available insulin preparations are characterized by a fast onset of action and other preparations have a relatively slow onset but show a more or less prolonged action. Fast acting insulin preparations are usually solutions of insulin, while retarded acting insulin preparations can be suspensions containing insulin in crystalline and/or amorphous form precipitated by addition of zinc salts alone or by addition of protamine or by a combination of both. In addition, some patients are using preparations having both a fast onset of action and a more prolonged action. Such a preparation may be an insulin solution wherein protamine insulin crystals are suspended. Some patients do themselves prepare the final preparation by mixing an insulin solution with a suspension preparation in the ratio desired by the patient in question.

Normally, insulin preparations are administered by subcutaneous injection. What is important for the patient, is the action profile of the insulin preparation which is the action of insulin on the glucose metabolism as a function of the time from the injection. In this profile, inter alia, the time for the onset, the maximum value and the total duration of action are important. A variety of insulin preparations with different action profiles are desired and requested by the patients. One patient may, on the same day, use insulin preparations with very different action profiles. The action profile requested is, for example, depending on the time of the day and the amount and composition of any meal eaten by the patient.

Equally important, however, is the physical stability of the insulin preparations due to the abundant use of pen-like injection devices such as devices which contain Penfill® cartridges, in which an insulin preparation is stored until the entire cartridge is empty. This may last for at least 1 to 2 weeks for devices containing 1.5-3.0 ml cartridges.

Description of the background art

The first stable neutral insulin suspension was developed by Scott and Fischer (J. Pharmacol. Exp. Ther. 58 (1936), 78) who discovered that the presence of a surplus of protamine and a zinc salt (2 µg zinc per IU (international Unit) insulin) could stabilize the protamine insulin preparation, described by Hagedorn et al.: J.Am.Med.Assn. 106 (1936), 177 - 180.

Protamine Zinc Insulin made according to the United States or European Pharmacopoeias contains amorphous protamine zinc insulin as well as crystalline Protamine Zinc Insulin. Freshly prepared protamine zinc insulin contains mainly amorphous precipitate which will partly be transformed into crystalline particles upon storage, leading to a more protracted effect.

A completely crystalline protamine zinc insulin modification designated NPH insulin or Isophane Insulin was developed by Krayenbühl and Rosenberg (see Rep. Steno Mem. Hosp. Nord. Insulinlab. 1 (1946), 60; and Danish patent No. 64,708). They found that insulin and protamine brought together in isophane proportions at a neutral pH value in the presence of a small amount of zinc and phenol, or phenol derivatives or, preferably m-cresol, will form an amorphous precipitate which upon standing is gradually but completely transformed into

oblong tetragonal crystals limited at the ends by pyramidal faces. Insulin and salmon protamine co-crystallize in a weight ratio corresponding to about 0.09 mg protamine sulphate per mg insulin. Zinc in an amount of at least 0.15 µg per IU and a phenolic in a concentration higher than 0.1% is necessary for the preparation of the tetragonal crystals.

In the early days, this kind of crystals were prepared using, porcine and bovine insulin from natural sources, but from the eighties also human insulin, made by genetic engineering or by semisynthesis, is used.

Human insulin consists of two polypeptide chains, the so-called A and B chains which contain 21 and 30 amino acids, respectively. The A and B chains are interconnected by two cystine disulphide bridges. Insulin from most other species has a similar construction, but may not contain the same amino acids at the positions corresponding in the chains as in human insulin.

The development of the process known as genetic engineering has made it possible easily to prepare a great variety of insulin compounds being analogous to human insulin. In these insulin analogues, one or more of the amino acids have been substituted with other amino acids which can be coded for by the nucleotide sequences. As human insulin, as explained above, contains 51 amino acid residues, it is obvious that a large number of insulin analogues is possible and, in fact, a great variety of analogues with interesting properties have been prepared. In human insulin solutions with a concentration of interest for injection preparations, the insulin molecule is present in associated form as a hexamer (Brange et al. Diabetes Care 13, (1990), 923 - 954). After subcutaneous injection, it is believed that the rate of absorption by the blood stream is dependent of the size of the molecule, and it has been found that insulin analogues with amino acid substitutions which counteract or inhibit this hexamer formation have an unusual fast onset of action (Brange et al.: Ibid). This is of great therapeutic value for the diabetic patient. In the crystals of the prolonged acting protamine insulin preparations, the insulin is also found to be hexameric (Balschmidt et al.: Acta Chryst. B47, (1991), 975 - 986).

Pharmaceutical preparations which are based on analogues of human insulin are e.g. known from the following documents:

WO 95/00550 is concerned with pharmaceutical preparations based on insulin crystals comprising Asp^{B28} insulin and protamine, which display rapid onset and prolonged activity when administered in vivo. The crystals may furthermore contains zinc ions and phenol and/or m-cresol. Glycerol is added to the preparations as isotonicity agent.

In US 5 461 031 various parenteral pharmaceutical formulations, which comprise a rapid-acting monomeric insulin analogue, zinc, protamine and a phenolic derivative, is disclosed. The formulations furthermore contain glycerol which acts as an isotonicity agent.

US 5 474 978 discloses a rapid acting parenteral formulation comprising a human insulin analogue hexamer complex consisting of six monomeric insulin analogues, zinc ions and at least three molecules of a phenolic derivative. The preferred isotonicity agent is glycerol.

Unfortunately, insulin tends to form insoluble and biologically inactive fibrils by non-covalent polymerization (c.f. e.g. Jens Brange, Galenics of Insulin, Springer-Verlag, 1987 and references therein). Fibril formation is promoted by elevated temperatures, e.g. above 30°C, and concomitant movements. This fibrillation process, which is very difficult to avoid, poses an upper limit on the period for which the insulin preparation can be stored and, hence, on the cartridge volume of Penfills®.

Since the formation of fibrils generally requires a monomerization of insulin, insulin analogues which more reluctantly form di- and hexamers, result in preparations of less physical stability due to fibrillation.

Thus, it is an object of the present invention to provide an insulin preparation comprising human insulin or an analogue or derivative thereof with improved physical stability.

According to the invention this object has been accomplished by an aqueous insulin preparation comprising dissolved and/or precipitated human insulin or an analogue or derivative thereof, and a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

Brief description f the drawing

Fig 1 is a photomicrograph (x1000 magnification) of a formulation of the invention comprising Asp^{B28} human insulin-protamine crystals and mannitol.

Fig 2 is a photomicrograph (x1000 magnification) of a formulation of the invention comprising human insulin-protamine crystals and mannitol.

Fig 3 is a graphical representation of the profile of action of a preparation of the invention containing both dissolved and crystalline Asp^{B28} human insulin and a preparation containing both dissolved and crystalline human insulin. Both preparations furthermore contain mannitol. The graph is the blood glucose response after injection in pigs. The figure demonstrate that the rapid onset of action is preserved for the highly stable Asp^{B28} human insulin preparation.

Description of the invention

Definitions

By "analogue of human insulin" as used herein is meant human insulin in which one or more amino acids have been deleted and/or replaced by other amino acids, including non-codeable amino acids, or human insulin comprising additional amino acids, i.e. more than 51 amino acids.

By "derivative of human insulin" as used herein is meant human insulin or an analogue thereof in which at least one organic substituent is bound to one or more of the amino acids.

In the present context the term "water-soluble" corresponds to a solubility in water of at least about 10 mmol/l, preferably at least 50 mmol/l, at a temperature of 20°C.

The terms carbohydrate, reduced carbohydrate, monosaccharide, disaccharide, and ester and ether derivatives of such compounds are used in accordance with the teaching of K. A. Jensen in "Grundrids af den organiske kemi, Almen Kemi III, 1. Ed., Jul. Gjellerups forlag, 1969, pp. 299-316.

In the present context the expression "non-reducing carbohydrate" denotes a carbohydrate which essentially is incapable of reacting with the amino groups of insulin in the preparations of the invention to form glycated insulin. This definition includes carbohydrates in which the carbonyl group(s) has/have been inactivated or blocked, e.g. by anhydride formation or derivatization.

In one aspect, the present invention relates to an aqueous insulin preparation comprising:

dissolved and/or precipitated human insulin or an analogue and/or a derivative thereof, and

100 to 400 mM, preferably 150 to 250 mM, more preferably 180 to 230 mM, of a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

In another aspect, the present invention relates to an aqueous insulin preparation comprising:

dissolved and precipitated human insulin or an analogue and/or a derivative thereof, and

a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

In yet another aspect, the present invention relates to an aqueous insulin analogue preparation comprising:

a dissolved and/or precipitated analogue of human insulin, and

a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

Preferred embodiments

The carbohydrate or carbohydrate derivative used in the insulin preparation of the invention advantageously contains from 5 to 18 carbon atoms in the main carbohydrate structure and is preferably selected from the following compounds:

i) monosaccharides selected from the group of non-reducing aldoses or ketoses, preferably non-reducing aldotetroses, ketotetroses, aldopentoses, ketopentoses, aldohexoses and ketohexoses, more preferably non-reducing aldopentoses, ketopentoses, aldohexoses and ketohexoses;

ii) reduced monosaccharides, i.e. polyhydric alcohols such as alditols, preferably selected from the group consisting of the reduced forms of aldotetroses, ketotetroses, aldopentoses, ketopentoses, aldohexoses and ketohexoses, more preferably of the reduced forms of aldopentoses, ketopentoses, aldohexoses and ketohexoses (i.e. pentitols and hexitols);

iii) non-reducing disaccharides, preferably selected from the non-reducing dihexoses.

Specific examples of suitable reduced or non-reducing carbohydrates are: mannitol, sorbitol, xylitol, inositol, sucrose and trehalose

The preferred compounds i) to iii) are mannitol and sorbitol, the most preferred being mannitol.

The preferred ester and ether derivatives are C_1-C_4 fatty acid ester derivatives and C_1-C_4 alkyl ether derivatives, respectively.

In a particular embodiment of the invention, the insulin preparation further comprises a halogenide, preferably a chloride, more preferably sodium chloride. It has been shown that the presence of a halogenide results in preparations having an even higher physical stability.

Since insulin preparations comprising fast-acting analogues of human insulin generally show a rather low physical stability, the present invention is particularly advantageous in connection with preparations comprising such analogues. Thus, the insulin preparation according to the invention preferably comprises one or more fast-acting analogues of human insulin, in particular analogues wherein position B28 is Asp, Lys, Leu, Val or Ala and position B29 is

Lys or Pro; or des(B28-B30), des(B27) or des(B30) human insulin. The insulin analogue is preferably selected from analogues of human insulin wherein position B28 is Asp or Lys, and position B29 is Lys or Pro. The most preferred analogues are Asp^{B28} human insulin or $Lys^{B28}Pro^{B29}$ human insulin.

In another embodiment the insulin preparation according to the invention comprises an insulin derivative having a protracted profile of action such as insulins having one or more lipophilic substituents. The preferred lipophilic insulins are acylated insulins, including those described in WO 95/07931 (Novo Nordisk A/S), e.g. human insulin derivatives wherein the ε-amino group of Lys^{B29} contains an acyl substituent which comprises at least 6 carbon atoms.

The preferred insulin derivatives are the following:

B29-N^{ε}-myristoyl-des(B30)-human insulin, B29-N^{ε}-myristoyl human insulin, B29-N^{ε}-palmitoyl human insulin, B28-N^{ε}-myristoyl Lys^{B28} Pro^{B29} human insulin, B28-N^{ε}-palmitoyl Lys^{B28} Pro^{B29} human insulin, B30-N^{ε}-myristoyl-$Thr^{B29}Lys^{B30}$-human insulin, B30-N^{ε}-palmitoyl-$Thr^{B29}Lys^{B30}$-human insulin, B29-N^{ε}-(N-palmitoyl-γ-glutamyl)-des(B30)-human insulin, B29-N^{ε}-(N-lithocholyl-γ-glutamyl)-des(B30)-human insulin and B29-N^{ε}-(ω-carboxyheptadecanoyl)-des(B30)-human insulin; the most preferred being B29-N^{ε}-myristoyl-des(B30)-human insulin.

In a preferred embodiment the insulin preparation comprises both dissolved and precipitated, preferably crystalline, insulin or insulin analogue or derivative in a weight ratio of 1:99 to 99:1, preferably 20:80 to 80:20, more preferably 30:70 to 70:30.

In this embodiment of the invention, the insulin preparation advantageously comprises crystals comprising: insulin or insulin analogue and protamine, and optionally zinc and/or a phenolic compound, such as phenol, m-cresol or a mixture thereof. The amount of protamine in the crystals preferably corresponds to 0.20 to 0.40 mg protamine base/100 IU insulin or insulin analogue. The ratio of protamine to insulin in the crystals more preferably corresponds to the isophane ratio. Zinc is preferably present in an amount of 10 to 40 μg Zn/100 IU insulin, more preferably 15 to 35 μg Zn/100 IU insulin. Phenol and m-cresol, respectively, is

preferably present in an amount corresponding to 0 to 4 mg/ml. However, a mixture of 1.4 to 2.0 mg/ml m-cresol and 0.6 to 2.0 mg/ml phenol is most preferred.

A preferred insulin preparation of the invention comprises:

a) 60 to 3000 nmol/ml, preferably 240 to 1200 nmol/ml, of human insulin or insulin analogue and/or insulin derivative;

b) a reduced or non-reducing carbohydrate, preferably mannitol, in a concentration of 100 to 400 mM, preferably 150 to 250 mM, more preferably 180 to 230 mM;

c) a chloride, preferably sodium chloride, in a concentration of 0 to 100 mM, preferably 5 to 40 mM, more preferably 5 to 20 mM; and

d) a physiologically tolerated buffer, preferably a phosphate buffer such as disodiumphosphatedihydrate in an amount of 1 to 4 mg/ml.

The preparation of the invention may furthermore contain one or more compounds commonly used as isotonicity agents, such as glycerol.

The pH value of the insulin preparation is preferably in the range 7.0 to 7.8.

The present invention is furthermore concerned with a method for preparing an insulin preparation containing both dissolved and precipitated insulin analogue, which method comprises the following steps:

a) providing an acidic solution comprising an analogue of human insulin, zinc, and a subisophanic amount of protamine;

b) providing an alkaline solution comprising a substance which acts as a buffer at physiological pH;

wherein at least one of the above solutions further comprises a phenolic compound;

c) mixing the acidic and alkaline solutions and, optionally, adjusting the pH to a value in the range of 6.5 to 8.0, preferably 7.0 to 7.8; and

d) leaving the resulting suspension for precipitation.

By this method an insulin preparation containing both dissolved and precipitated insulin analogue can be obtained in a very simple manner. Furthermore, the precipitate of the resulting suspension usually consists of rod-shaped crystals, which are advantageous in so-called PreMix insulin preparations.

The weight ratio of insulin analogue to protamine in the solution of step a) is preferably selected so as to obtain in the final product a weight ratio of dissolved to precipitated insulin analogue in the range of 1:99 to 99:1, preferably 20:80 to 80:20, more preferably 30:70 to 70:30. More specifically, the solution of step a) preferably comprises 120 to 6000 nmol/ml of insulin analogue and 0.01 to 5.0 mg/ml of protamine.

The solution of step a) further comprises zinc, preferably in an amount corresponding to 10 - 40 ug zinc/100 IU insulin, more preferably 15 - 35 ug zinc/100 IU insulin.

In a preferred embodiment, solution a) and/or solution b) comprises chloride, preferably sodium chloride, in an amount corresponding to 0 to 100 mM, preferably 5 to 40 mM, more preferably 5 to 20 mM, in the final product.

The pH of the acidic solution of step a) is preferably below 5, more preferably in the range of 2 to 3.5.

The insulin analogue is preferably human insulin wherein position B28 is Asp, Lys, Leu, Val or Ala and position B29 is Lys or Pro; or des(B28-B30), des(B27) or des(B30) human insulin, more preferably Asp^{B28} human insulin or $Lys^{B28}Pro^{B29}$ human insulin, still more preferably Asp^{B28} human insulin.

The phenolic compound used in the solution of step a) and/or step b) is preferably phenol, m-cresol or a mixture thereof.

Advantageously, the solution of step a) and/or step b) further comprises a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main

carbohydrate structure, or mixtures thereof.

Said carbohydrate or carbohydrate derivative preferably contains from 5 to 18 carbon atoms in the main carbohydrate structure.

In a particular preferred embodiment, the solution of step a) and/or step b) comprises mannitol, sorbitol, xylitol, inositol, trehalose, sucrose or any mixture thereof, preferably mannitol and/or sorbitol, more preferably mannitol.

The buffer substance employed in the alkaline solution of step b) is preferably a physiologically tolerated buffer, preferably a phosphate buffer, more preferably disodium phosphate dihydrate.

The precipitated insulin analogue is preferably in the form of crystals comprising insulin analogue and protamine.

The suspension obtained in step d) is preferably left at a temperature in the range of 5°C to 40°C, more preferably 20°C to 36°C, still more preferably 30°C to 34°C, for precipitation.

This invention is further illustrated by the following examples which, however, are not to be construed as limiting.

EXAMPLE I

Preparation 1

An insulin preparation containing both dissolved and crystalline Asp^{B28} human insulin was prepared in the following way:

Solution A:

A solution of Asp^{B28} human insulin at 200 IU/ml concentration was prepared by dissolving 76.5 mg of Asp^{B28} human insulin in water by adding to it 326 µl 0.2 N hydrochloric Acid and 163 µl zinc chloride solution (0.4 mg/ml). Then 6.35 mg protamine sulphate in solution

was added to the insulin solution while mixing, and a mixture consisting of 17.2 mg m-cresol, 15 mg phenol and 455 mg mannitol was added to this solution while mixing. The pH of the resulting clear solution was measured to pH=2.6-2.9, and water ad 10 ml was added. The solution was equilibrated at 28-32°C.

Solution B:

25 mg disodium phosphate dihydrate was dissolved in Water for Injection. 17.2 mg m-cresol, 15 mg phenol and 455 mg mannitol was added during mixing. The pH of the resulting clear solution was measured to 9, and water ad 10 ml was added. The solution was equilibrated at 28-32°C.

Mixing of solution A and B:

Solution B was added to solution A, and the pH was readjusted to 7.30. The resulting suspension was left at 30°C for 6 days for crystallisation.

In the resulting preparation, the weight ratio of precipitated to dissolved insulin was 70:30.

The preparation was introduced into 1.5 ml Penfill® cartridges.

EXAMPLE II

Preparation 2

An insulin preparation containing both dissolved and crystalline Asp^{B28} human insulin was prepared in the following way:

i) Crystalline fraction.

Solution A:

A solution of Asp^{B28} human insulin at 200 IU/ml concentration was prepared by dissolving 190.3 mg of Asp^{B28} human insulin in water by adding to it 813 µl 0.2 N hydrochloric acid

and 410 µl zinc chloride solution (0.4 mg/ml). Then 16.1 mg protamine sulphate in solution was added to the insulin solution while mixing, and a mixture consisting of 43.0 mg m-cresol, 37.5 mg phenol and 909 mg mannitol, and 14.6 mg sodium chloride was added to this solution while mixing. The pH of the resulting clear solution was measured to pH=2.6-2.9, and water ad 22 ml was added. The solution was equilibrated at 32°C.

Solution B:

62.4 mg disodium phosphate dihydrate was dissolved in water. 43.0 mg m-cresol, 37.5 mg phenol, 909 mg mannitol, and 14.6 mg sodium chloride was added during mixing. The pH of the resulting clear solution was measured to 9, and water ad 22 ml was added. The solution was equilibrated at 32°C.

Mixing of solution A and B:

Solution B was added to solution A, and the pH was readjusted to 7.30, and water ad 50 ml was added. The resulting suspension was left at 32°C for 4 days for crystallization.

In the resulting preparation, the weight ratio of precipitated to dissolved insulin was 70:30.

The preparation was introduced into 1.5 ml Penfill® cartridges.

EXAMPLES III-VI

Preparations 3 to 6

Insulin preparations containing both dissolved and crystalline Asp^{B28} human insulin was prepared as described in Example II, except that the amount of mannitol used in Solution A and B was 818 mg, 1005 mg, 1047 mg and 1137 mg , respectively.

The preparations were introduced into 1.5 ml Penfill® cartridges.

EXAMPLE VII

Preparation 7

An insulin preparation containing both dissolved and crystalline $Lys^{B28}Pro^{B29}$ human insulin was prepared in the following way:

i) Crystalline fraction.

Solution A:

A solution of $Lys^{B28}Pro^{B29}$ human insulin at 200 IU/ml concentration was prepared by suspending 69.7 mg of $Lys^{B28}Pro^{B29}$ human insulin in water. A mixture consisting of 16.0 mg m-cresol, 6.5 mg phenol, 364 mg mannitol and 25.1 mg disodium diphosphate dihydrate was added to this solution while mixing. Then 50 µl zinc chloride solution (10 mg/ml) was added. The pH of the resulting clear solution was readjusted to 7.40, and water ad 10 ml was added. The solution was equilibrated at 15°C.

Solution B:

A solution of protamine sulphate was prepared by dissolving 7.61 mg protamine sulphate and 25.1 mg disodium phosphate dihydrate in water. 16.0 mg m-cresol, 6.5 mg phenol and 364 mg mannitol was added during mixing. This solution was added to the protamine sulphate solution while mixing. The pH of the resulting clear solution was readjusted to 7.40, and water ad 10 ml was added. The solution was equilibrated at 15°C.

Mixing of solution A and B:

Solution B was added to solution A, and the pH was readjusted to 7.30. The resulting suspension was left at 15°C for 3 days for crystallization.

ii) Dissolved fraction

A solution of $Lys^{B28}Pro^{B29}$ human insulin was prepared by dissolving 34.9 mg $Lys^{B28}Pro^{B29}$

human insulin in water by adding to it 33 µl 1 N hydrochloric acid and 25 µl zinc chloride solution (10 mg/ml). Then a mixture consisting of 26.1 mg sodium phosphate dihydrate, 6.5 mg phenol, 16.0 mg m-cresol and 364 mg mannitol was added to the insulin solution while mixing. The pH of the resulting clear solution was measured to pH = 7.3, and water ad 10 ml was added.

6 ml of the dissolved fraction was added to 14 ml of the crystalline fraction, and the pH was adjusted to 7.30.

In the resulting preparation, the weight ratio of precipitated to dissolved insulin was 70:30.

The preparation was introduced into 1.5 ml Penfill® cartridges.

EXAMPLE VIII

Preparation 8

An insulin preparation containing both dissolved and crystalline human insulin was prepared in the following way:

i) Crystalline fraction

Solution A:

A solution of human insulin was prepared by dissolving 69.7 mg human insulin in water by adding to it 65 µl 1 N hydrochloric acid and 26 µl zinc chloride solution (10 mg/ml). Then 6.0 mg protamine sulphate in solution was added to the insulin solution while mixing, and a mixture consisting of 15.0 mg phenol and 17.2 mg m-cresol was added to the solution while mixing. The pH of the resulting clear solution was measured to pH = 2.7 - 3.2, and water ad 10 ml was added.

Solution B:

24.9 mg sodium phosphate dihydrate was dissolved in water. 15 mg phenol, 17.2 mg m-cresol, 728 mg mannitol and 11.7 mg sodium chloride was added during mixing. The pH of

the resulting clear solution was adjusted to pH = 9, and water ad 10 ml was added.

Solution B was added to solution A, and the pH was adjusted to 7.30. The resulting suspension was left at 22-24 °C until the next day.

ii) Dissolved fraction

A solution of human insulin was prepared by dissolving 34.9 mg human insulin in water by adding to it 33 µl 1 N hydrochloric acid and 13 µl zinc chloride solution (10 mg/ml). Then a mixture consisting of 12.5 mg sodium phosphate dihydrate, 15 mg phenol, 17.2 mg m-cresol, 364 mg mannitol and 5.8 mg sodium chloride was added to the insulin solution while mixing. The pH of the resulting clear solution was measured to pH = 7.3, and water ad 10 ml was added.

6 ml of the dissolved fraction was added to 14 ml of the crystalline fraction, and the pH was adjusted to 7.30.

In the resulting preparation, the weight ratio of precipitated to dissolved insulin was 70:30.

The preparation was introduced into 1.5 ml Penfill® cartridges.

EXAMPLE IX

Preparation 9

An insulin preparation containing both dissolved and crystalline Asp^{B28} human insulin was prepared in the following way:

Solution A:

A solution of Asp^{B28} human insulin at 200 IU/ml concentration was prepared by dissolving 189.9 mg of Asp^{B28} human insulin in water by adding to it 163 µl 1 N hydrochloric acid and 163.5 µl zinc chloride solution (10 mg/ml). Then 11.5 mg protamine sulphate in solution was added to the insulin solution while mixing, and a mixture consisting of 44.3 mg m-

cresol, 38.6 mg phenol and 1048 mg mannitol[1], and 7.3 mg sodium chloride was added to this solution while mixing. The pH of the resulting clear solution was measured to pH=2.6-2.9, and water ad 25 ml was added. The solution was equilibrated at 22-24°C.

Solution B:

62.3 mg disodium phosphate dihydrate was dissolved in water. 44.3 mg m-cresol, 38.6 mg phenol, 1048 mg mannitol, and 7.3 mg sodium chloride was added during mixing. The pH of the resulting clear solution was measured to 9, and water ad 25 ml was added. The solution was equilibrated at 22-24°C.

Mixing of solution A and B:

Solution B was added to solution A, and the pH was readjusted to 7.30. The resulting suspension was left at 32°C for 2 days for crystallisation.

In the resulting preparation, the weight ratio of precipitated to dissolved insulin was 50:50.

The preparation was introduced into 1.5 ml Penfill® cartridges.

EXAMPLE X (Comparative)

Preparation 10

An insulin preparation containing both dissolved and crystalline Asp^{B28} human insulin was prepared in the following way:

Solution A:

A solution of Asp^{B28} human insulin at 200 IU/ml concentration was prepared by dissolving 76.5 mg of Asp^{B28} human insulin in water by adding to it 326 µl 0.2 N hydrochloric acid and 163 µl zinc chloride solution (0.4 mg/ml). Then 6.35 mg protamine sulphate in solution was added to the insulin solution while mixing, and a mixture consisting of 17.2 mg m-cresol, 15.0 mg phenol and 160 mg glycerol was added to this solution while mixing. The

pH of the resulting clear solution was measured to pH=2.6-2.9, and water ad 10 ml was added. The solution was equilibrated at 28-32°C.

Solution B:

25 mg disodium phosphate dihydrate was dissolved in Water for Injection. 17.2 mg m-cresol, 15.0 mg phenol and 160 mg glycerol was added during mixing. The pH of the resulting clear solution was measured to 9, and water ad 10 ml was added. The solution was equilibrated at 28-32°C.

Solution B was added to solution A, and the pH was readjusted to 7.30. The resulting suspension was left at 28-32°C for 2 days for crystallization.

In the resulting preparation, the weight ratio of precipitated to dissolved insulin was 70:30.

The preparation was introduced into 1.5 ml Penfill® cartridges.

EXAMPLE XI

PHYSICAL STRESS TESTS

5 samples of each insulin preparation were introduced into Penfill® cartridges and subjected to the following physical stress test:

The Penfill® cartridges were fixed to a rotator which were placed in an incubator, and rotated 360° for four hours per day with a frequency of 30 rotations per minute and at a constant temperature of 37°C±2°C. The Penfill® cartridges were stored at a temperature of 37°C±2°C, when they were not rotated.

The Penfill® cartridges were inspected macroscopically 5 times a week and changes in the appearance of the formulation were noted according to following principles:21

i) Cartridges containing a white suspension resuspendable on agitation and free of lumps and granules were classified as "not fibrillated".

ii) Cartridges containing a suspension with lumps and/or granules not resuspendable on agitation and/or depositing on the cartridge wall were assumed "fibrillated". This was confirmed by addition of 6 µl 6N HCl to the cartridge: Fibrillated cartridges are not visually clear after addition of acid.

Rotation of the cartridges was continued until all samples were fibrillated.

The results are summarized in the following Table I

TABLE I

Preparation No.	Composition	Average No. of days to fibrillation	First day of fibrillation
1	Asp^{B28} insulin 30/70 7 mM phosphate 250 mM mannitol	20	20
2	Asp^{B28} insulin 30/70 7 mM phosphate 200 mM mannitol 10 mM sodium chloride	23	23
3	Asp^{B28} insulin 30/70 7 mM phosphate 180 mM mannitol 10 mM sodium chloride	21	17
4	Asp^{B28} insulin 30/70 7 mM phosphate 220 mM mannitol 10 mM sodium chloride	19	17
5	Asp^{B28} insulin 30/70 7 mM phosphate 230 mM mannitol 10 mM sodium chloride	21	18
6	Asp^{B28} insulin 30/70 7 mM phosphate 250 mM mannitol 10 mM sodium chloride	22	22
9	Asp^{B28} insulin 50/50 7 mM phosphate 230 mM mannitol 10 mM sodium chloride	>30	>30
10 (Comparative)	Asp^{B28} insulin 30/70 7 mM phosphate 174 mM glycerol	11	9

Claims

1. An aqueous insulin preparation comprising:

dissolved and/or precipitated human insulin or an analogue and/or a derivative thereof, and

100 to 400 mM, preferably 150 to 250 mM, more preferably 180 to 230 mM, of a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

2. An insulin preparation according to claim 1, wherein said carbohydrate or carbohydrate derivative contains from 5 to 18 carbon atoms in the main carbohydrate structure.

3. An insulin preparation according to claim 2, comprising mannitol, sorbitol, xylitol, inositol, trehalose, sucrose or any mixture thereof, preferably mannitol and/or sorbitol, more preferably mannitol.

4. An insulin preparation according to any of the preceding claims, which further comprises a halogenide, preferably a chloride, more preferably sodium chloride.

5. An insulin preparation according to any of the preceding claims, comprising an analogue of human insulin wherein position B28 is Asp, Lys, Leu, Val or Ala and position B29 is Lys or Pro; or des(B28-B30), des(B27) or des(B30) human insulin.

6. An insulin preparation according to claim 5, comprising an analogue of human insulin wherein position B28 is Asp or Lys, and position B29 is Lys or Pro, preferably Asp^{B28} human insulin or $Lys^{B28}Pro^{B29}$ human insulin.

7. An insulin preparation according to any one of claims 1 to 4, comprising a derivative of human insulin having one or more lipophilic substituents, preferably an acylated insulin.

8. An insulin preparation according to claim 7, comprising a derivative of human insulin

selected from the group consisting of:

B29-N$^{\varepsilon}$-myristoyl-des(B30)-human insulin, B29-N$^{\varepsilon}$-myristoyl human insulin, B29-N$^{\varepsilon}$-palmitoyl human insulin, B28-N$^{\varepsilon}$-myristoyl Lys^{B28} Pro^{B29} human insulin, B28-N$^{\varepsilon}$-palmitoyl Lys^{B28} Pro^{B29} human insulin, B30-N$^{\varepsilon}$-myristoyl-Thr^{B29}Lys^{B30}-human insulin, B30-N$^{\varepsilon}$-palmitoyl-Thr^{B29}Lys^{B30}-human insulin, B29-N$^{\varepsilon}$-(N-palmitoyl-γ-glutamyl)-des(B30)-human insulin, B29-N$^{\varepsilon}$-(N-lithocholyl-γ-glutamyl)-des(B30)-human insulin and B29-N$^{\varepsilon}$-(ω-carboxyheptadecanoyl)-des(B30)-human insulin; preferably B29-N$^{\varepsilon}$-myristoyl-des(B30)-human insulin.

9. An insulin preparation according to any of the preceding claims, which comprises crystals comprising human insulin or an analogue or derivative thereof and protamine.

10. An insulin preparation according to claim 9, in which the crystals further comprises zinc and optionally a phenolic compound, preferably phenol, m-cresol or a mixture thereof.

11. An insulin preparation according to any of the preceding claims comprising dissolved and precipitated, preferably crystalline, insulin or insulin analogue in a weight ratio of 1:99 to 99:1, preferably 20:80 to 80:20, more preferably 30:70 to 70:30.

12. An insulin preparation according to any one of the preceding claims, comprising:

60 to 3000 nmol/ml, preferably 240 to 1200 nmol/ml of human insulin or insulin analogue or derivative.

13. An insulin preparation according to any one of the preceding claims, comprising:

0 to 100 mM, preferably 5 to 40 mM, more preferably 5 to 20 mM, of a chloride, preferably sodium chloride.

14. An insulin preparation according to any one of the preceding claims, further comprising

a physiologically tolerated buffer, preferably a phosphate buffer.

15. An aqueous insulin preparation comprising:

dissolved and precipitated human insulin or an analogue and/or a derivative thereof, and

a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

16. An insulin preparation according to claim 15, wherein said carbohydrate or carbohydrate derivative contains from 5 to 18 carbon atoms in the main carbohydrate structure.

17. An insulin preparation according to claim 16, comprising mannitol, sorbitol, xylitol, inositol, trehalose, sucrose or any mixture thereof, preferably mannitol and/or sorbitol, more preferably mannitol.

18. An insulin preparation according to any of the claims 15 to 17, which further comprises a halogenide, preferably a chloride, more preferably sodium chloride.

19. An insulin preparation according to any of the claims 15 to 18, comprising an analogue of human insulin wherein position B28 is Asp, Lys, Leu, Val or Ala and position B29 is Lys or Pro; or des(B28-B30), des(B27) or des(B30) human insulin.

20. An insulin preparation according to claim 19, comprising an analogue of human insulin wherein position B28 is Asp or Lys, and position B29 is Lys or Pro, preferably Asp^{B28} human insulin or $Lys^{B28}Pro^{B29}$ human insulin.

21. An insulin preparation according to any one of claims 15 to 18, comprising a derivative of human insulin having one or more lipophilic substituents, preferably an acylated insulin.

22. An insulin preparation according to claim 21, comprising a derivative of human insulin selected from the group consisting of:

B29-N^{ε}-myristoyl-des(B30)-human insulin, B29-N^{ε}-myristoyl human insulin, B29-N^{ε}-palmitoyl human insulin, B28-N^{ε}-myristoyl Lys^{B28} Pro^{B29} human insulin, B28-N^{ε}-palmitoyl Lys^{B28} Pro^{B29} human insulin, B30-N^{ε}-myristoyl-$Thr^{B29}Lys^{B30}$-human insulin, B30-N^{ε}-palmitoyl-$Thr^{B29}Lys^{B30}$-human insulin, B29-N^{ε}-(N-palmitoyl-γ-glutamyl)-

des(B30)-human insulin, B29-N$^{\varepsilon}$-(N-lithocholyl-γ-glutamyl)-des(B30)-human insulin and B29-N$^{\varepsilon}$-(ω-carboxyheptadecanoyl)-des(B30)-human insulin; preferably B29-N$^{\varepsilon}$-myristoyl-des(B30)-human insulin.

23. An insulin preparation according to any of the claims 15 to 22, which comprises crystals comprising insulin, insulin analogue or insulin derivative, and protamine.

24. An insulin preparation according to claim 23, in which the crystals further comprises zinc and optionally a phenolic compound, preferably phenol, m-cresol or a mixture thereof.

25. An insulin preparation according to any of the claims 15 to 24, comprising dissolved and precipitated, preferably crystalline, insulin or insulin analogue in a weight ratio of 1:99 to 99:1, preferably 20:80 to 80:20, more preferably 30:70 to 70:30.

26. An insulin preparation according to any of the claims 15 to 25, in which the reduced or non-reducing carbohydrate, preferably mannitol, is present in an amount corresponding to 100 to 400 mM, preferably 150 to 250 mM, more preferably 180 to 230 mM.

27. An insulin preparation according to any one of the claims 13 to 26, comprising:

60 to 3000 nmol/ml, preferably 240 to 1200 nmol/ml of human insulin or insulin analogue or derivative.

28. An insulin preparation according to any one of the claims 15 to 27, comprising:

0 to 100 mM, preferably 5 to 40 mM, more preferably 5 to 20 mM, of a chloride, preferably sodium chloride.

29. An insulin preparation according to any one of the claims 15 to 29, further comprising

a physiologically tolerated buffer, preferably a phosphate buffer.

30. An aqueous insulin analogue preparation comprising:

a dissolved and/or precipitated analogue of human insulin, and

a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

31. An insulin preparation according to claim 30, wherein said carbohydrate or carbohydrate derivative contains from 5 to 18 carbon atoms in the main carbohydrate structure.

32. An insulin preparation according to claim 31, comprising mannitol, sorbitol, xylitol, inositol, trehalose, sucrose or any mixture thereof, preferably mannitol and/or sorbitol, more preferably mannitol.

33. An insulin preparation according to any one of the claims 30 to 32, which further comprises a halogenide, preferably a chloride, more preferably sodium chloride.

34. An insulin preparation according to any one of the claims 30 to 33, wherein the analogue of human insulin is human insulin wherein position B28 is Asp, Lys, Leu, Val or Ala and position B29 is Lys or Pro; or des(B28-B30), des(B27) or des(B30) human insulin.

35. An insulin preparation according to claim 34, wherein the analogue of human insulin is human insulin wherein position B28 is Asp or Lys, and position B29 is Lys or Pro, preferably Asp^{B28} human insulin or $Lys^{B28}Pro^{B29}$ human insulin.

36. An insulin preparation according to claim 35, wherein the analogue of human insulin is Asp^{B28} human insulin.

37. An insulin preparation according to any one of the claims 30 to 36, which comprises crystals comprising insulin analogue and protamine.

38. An insulin preparation according to claim 37, in which the crystals further comprises zinc and optionally a phenolic compound, preferably phenol, m-cresol or a mixture thereof.

39. An insulin preparation according to any one of the claims 30 to 38, comprising dissolved and precipitated, preferably crystalline, insulin analogue in a weight ratio of 1:99 to 99:1, preferably 20:80 to 80:20, more preferably 30:70 to 70:30.

40. An insulin preparation according to any one of the claims 30 to 39, comprising:

60 to 3000 nmol/ml, preferably 240 to 1200 nmol/ml of an analogue of human insulin.

41. An insulin preparation according to any one of the claims 30 to 40, comprising:

0 to 100 mM, preferably 5 to 40 mM, more preferably 5 to 20 mM, of a chloride, preferably sodium chloride.

42. An insulin preparation according to any one of the claims 30 to 41, further comprising

a physiologically tolerated buffer, preferably a phosphate buffer.

43. A parenteral pharmaceutical formulation comprising an insulin preparation according to any of the preceding claims.

44. The use of a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof, for improving the physical stability of insulin preparations comprising human insulin or an analogue and/or a derivative thereof.

45. The use of mannitol, sorbitol, xylitol, inositol, trehalose, sucrose or any mixture thereof, preferably mannitol and/or sorbitol, more preferably mannitol, for improving the physical stability of insulin preparations comprising human insulin or an analogue and/or a derivative thereof.

46. A method for preparing an insulin preparation containing both dissolved and precipitated insulin analogue, comprising the steps of:

a) providing an acidic solution comprising an analogue of human insulin, zinc, and a sub-isophanic amount of protamine;

b) providing an alkaline solution comprising a substance which acts as a buffer at physiological pH;

wherein at least one of the above solutions further comprises a phenolic compound;

c) mixing the acidic and alkaline solutions and, optionally, adjusting the pH to a value in the range of 6.5 to 8.0, preferably 7.0 to 7.8; and

d) leaving the resulting suspension for precipitation.

47. A method according to claim 46, wherein the weight ratio of insulin analogue to protamine in the solution of step a) is selected so as to obtain in the final product a weight ratio of dissolved to precipitated insulin analogue in the range of 1:99 to 99:1, preferably 20:80 to 80:20, more preferably 30:70 to 70:30.

48. A method according to claim 47, wherein the solution of step a) comprises 120 to 6000 nmol/ml of insulin analogue and 0.01 to 5.0 mg/ml of protamine.

49. A method according to claim 48, wherein the solution of step a) further comprises zinc, preferably zinc chloride, in an amount corresponding to 10 - 40 µg zinc/100 IU insulin, preferably 15 - 35 µg zinc/100 IU insulin.

50. A method according to any one of the claims 46 to 49, wherein solution a) and/or solution b) comprises chloride, preferably sodium chloride, in an amount corresponding to 0 to 100 mM, preferably 5 to 40 mM, more preferably 5 to 20 mM, in the final product.

51. A method according to any one of the claims 46 to 50, wherein the pH of the acidic solution of step a) is below 5, preferably in the range of 2 to 3.5.

52. A method according to any one of the claims 46 to 51, wherein the insulin analogue is human insulin wherein position B28 is Asp, Lys, Leu, Val or Ala and position B29 is Lys or Pro; or des(B28-B30), des(B27) or des(B30) human insulin.

53. A method according to claim 52, wherein the insulin analogue is Asp^{B28} human insulin or $Lys^{B28}Pro^{B29}$ human insulin, preferably Asp^{B28} human insulin.

54. A method according to any one of the claims 46 to 53 wherein the solution of step a) and/or step b) further comprises a phenolic compound, preferably phenol, m-cresol or a mixture thereof.

55. A method according to any one of the claims 46 to 54 wherein the solution of step a) and/or step b) further comprises a water-soluble reduced or non-reducing carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or a water-soluble non-reducing ester and/or ether derivative of a carbohydrate or reduced carbohydrate containing at least 4 carbon atoms in the main carbohydrate structure, or mixtures thereof.

56. A method according to claim 55, wherein said carbohydrate or carbohydrate derivative contains from 5 to 18 carbon atoms in the main carbohydrate structure.

57. A method according to claim 56, wherein the solution of step a) and/or step b) comprises mannitol, sorbitol, xylitol, inositol, trehalose, sucrose or any mixture thereof, preferably mannitol and/or sorbitol, more preferably mannitol.

58. A method according to any one of the claims 46 to 57, wherein the buffer substance employed in the alkaline solution of step b) is a physiologically tolerated buffer, preferably a phosphate buffer, more preferably disodium phosphate dihydrate.

59. A method according to any one of the claims 46 to 58, wherein the precipitated insulin analogue is in the form of crystals comprising insulin analogue and protamine.

60. A method according to any one of the claims 46 to 59, wherein the suspension obtained in step d) is left at a temperature in the range of 5°C to 40°C, preferably 20°C to 36°C, more preferably 30°C to 34°C, for precipitation.

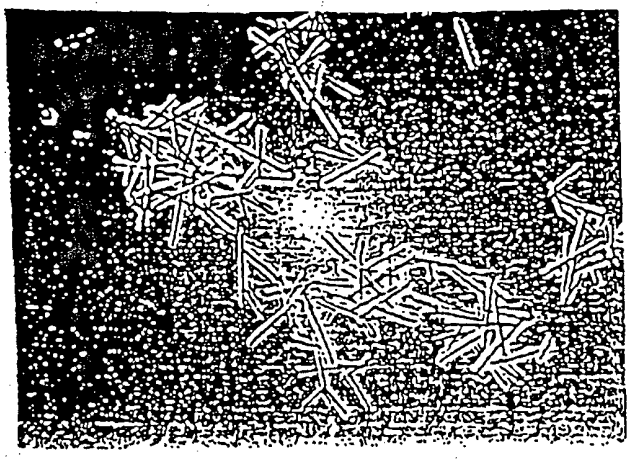

FIG. 1

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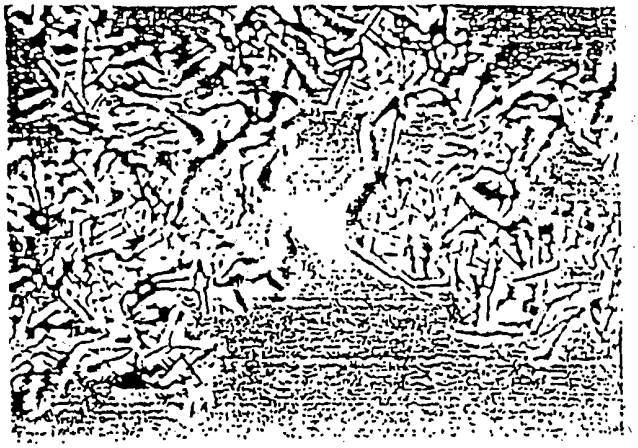

FIG. 2

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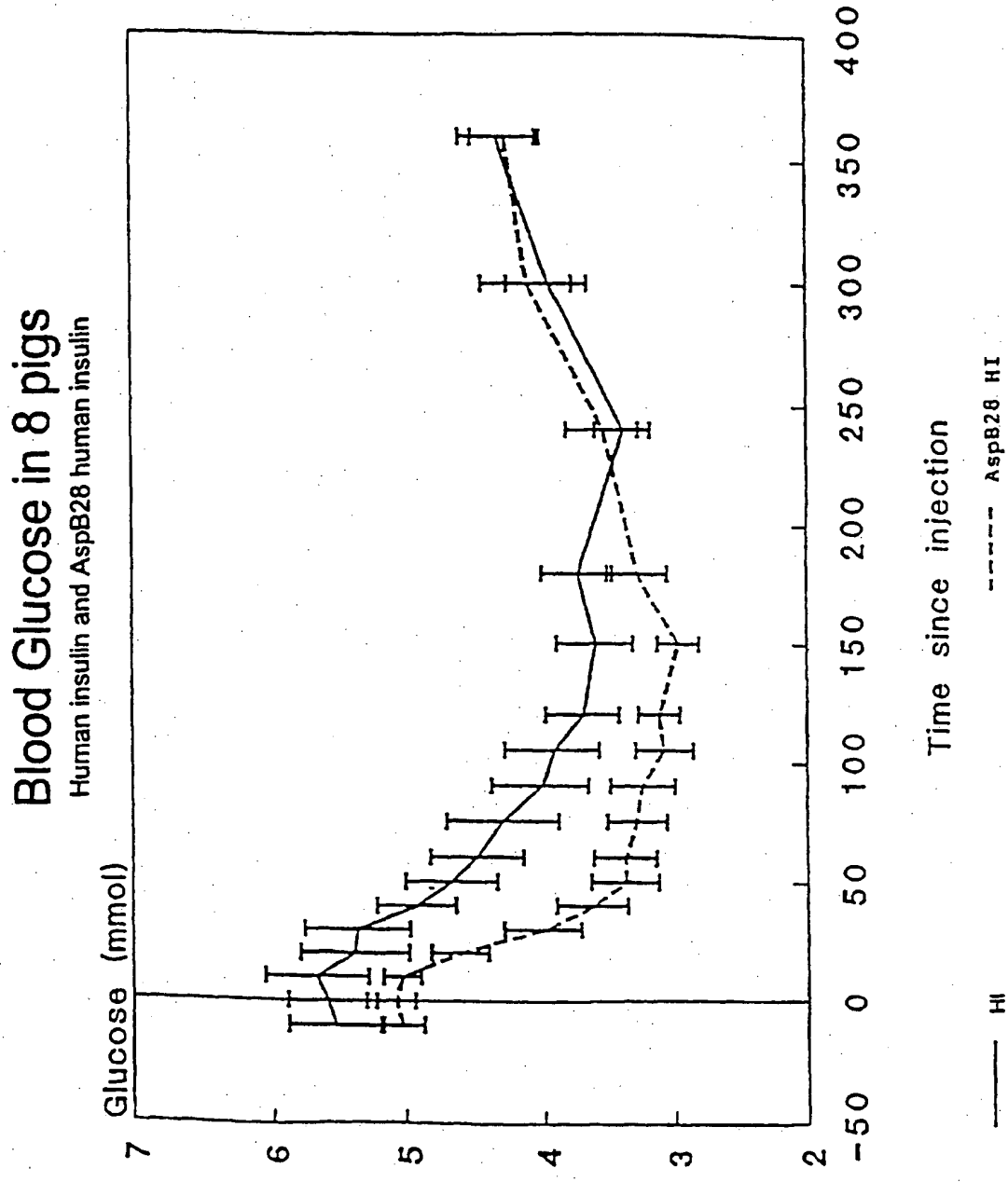

FIG. 3

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00267

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/28, A61K 47/26
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, EMBASE, WPI, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Centre for Biotechnology, vol. 70, Vinita Gupta et al: "Effect of solvent additives on the thermal stability of insulin", page 209 - page 212 --	1-3,5-17, 19-32,34-40, 42-49,51-60
X	US 4439181 A (PERRY J. BLACKSHEAR ET AL), 27 March 1984 (27.03.84) --	1-3,5-17, 19-32,34-40, 42-49,51-60
A	WO 9500550 A1 (NOVO NORDISK A/S), 5 January 1995 (05.01.95) --	1-60

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"P" document published prior to the international filing date but later than the priority date claimed
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Date of the actual completion of the international search	Date of mailing of the international search report
9 October 1997	17 -10- 1997
Name and mailing address of the ISA/ Swedish Patent Office B x 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/09/97

Inter. ional application No.
PCT/DK 97/00267

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US 4439181 A	27/03/84	NONE	
WO 9500550 A1	05/01/95	AU 6995794 A	17/01/95
		EP 0705275 A	10/04/96
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Form PCT/ISA/210 (patent family annex) (July 1992)

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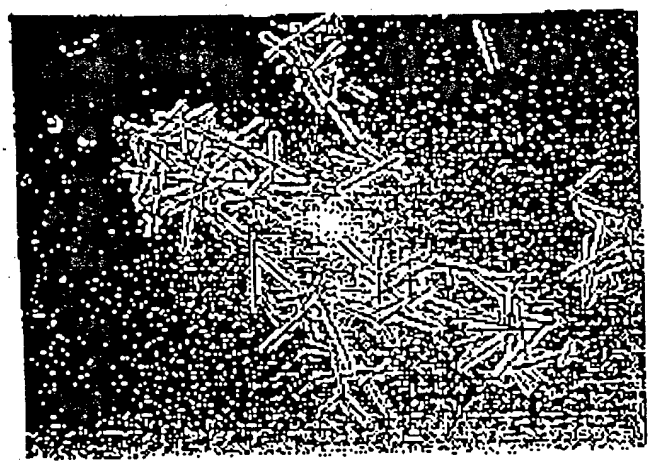

FIG. 1

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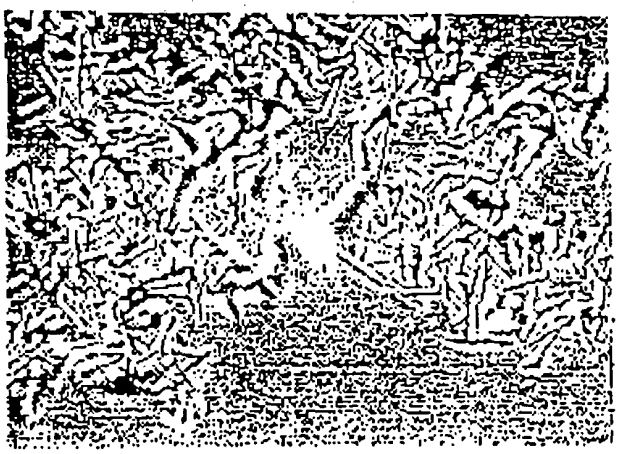

FIG. 2

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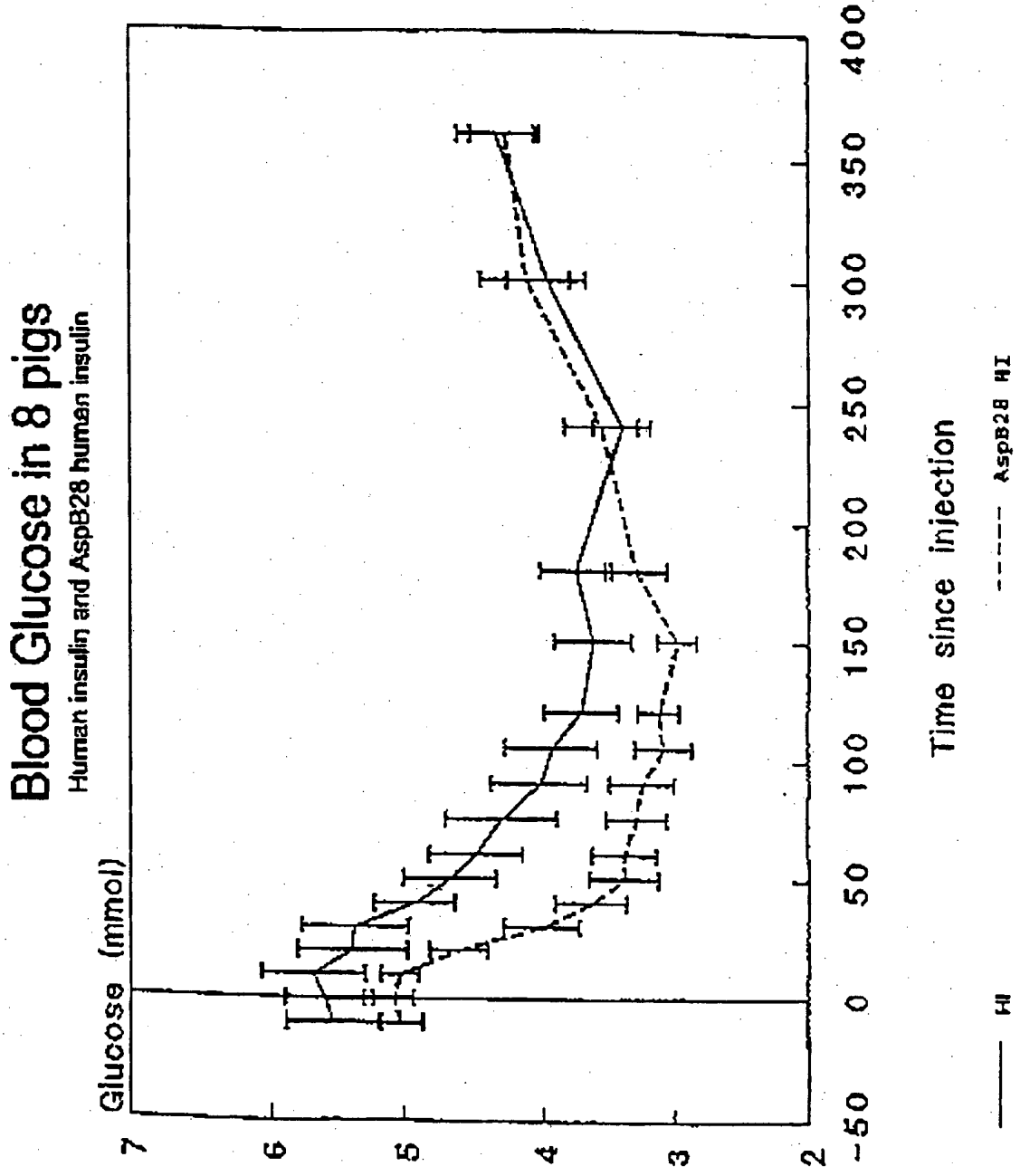

FIG. 3

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